Parasitoids of *Paratachardina lobata* (Hem., Kerriidae): surveys for biological control of the invasive lobate lac scale

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Abstract

Paratachardina lobata is an invasive pest in southern Florida, threatening a great number of economically important and native plants. The lobate lac scale does not cause problems in its area of origin, India and Sri Lanka, presumably due to various parasitoid wasps. In an attempt to discover promising parasitoids for biological control against the invasive pest in Florida, *P. lobata* infesting *Pongamia pinnata* (Fabaceae) was collected bimonthly from August 2005 to June 2006 at 14 sites in southern India. Four parasitoids were demonstrably associated with *P. lobata*: a eulophid *Aprostocetus bangaloricus* Narendran, an encyrtid *Ooencyrtus kerriae* Hayat and two aphelinids, *Marietta leopardina* Motschulsky and an undescribed *Coccophagus* Westwood sp. These chalcidoid wasps were found regularly at all heavily infested sites with an average emergence number per collection period of 62.8, 31.7, 11.9 and 90.5 respectively. The mean number emerged 20–28 days after the collection date, excluding the *Coccophagus* sp., which occurred significantly later, on average 41 days after collection. Species emergence was examined and parasitized scales dissected. Parasitoid remains were interpreted to understand the mode of parasitism. The *Coccophagus* sp. was found to be a secondary parasitoid. *Marietta leopardina* occurred as a primary parasitoid, but only in low number and this species is also known to be hyperparasitic on chalcidoid wasps. *Aprostocetus bangaloricus and O. kerriae* are promising candidates for the lobate lac scale control in Florida. They are primary parasitoids of *P. lobata* and occurred at almost every collection site, especially where *P. lobata* was very abundant.

Introduction

The lobate lac scale has invaded the Bahamas and southern Florida, where it threatens a great number of native and economic plants (Pemberton 2003a). The scale, identified as *Paratachardina lobata* (Chamberlin) (Hem., Kerriidae), was detected first in the Bahamas in 1992 and in southern Florida in 1999 (Howard and Pemberton 2003). It is highly polyphagous (Varshney 1992) and spreads very fast in southern Florida. The number of recorded hosts has snowballed since its invasion of more than 300 plant species (Howard et al. 2006). Although effective controls using insecticide oils and systemic pesticides have been developed (Howard and Steinberg 2005), applications of those insecticides are unsuitable for widespread use in the natural areas infested by lobate lac scales in Florida and the Bahamas. Therefore sustainable biological control strategies are urgently required to control this invasive pest. In southern Florida parasitism of the lobate lac scale is rare. Two encyrtids, *Ammonoencyrtus carolinensis* (N. Comb.), also known to attack the coccid *Mesolecanium nigrofasciatum* (Pergande), and an unnamed *Metaphycus* sp. are the only isolated parasitoids associated with lobate lac scales in Florida (Howard and

J. Appl. Entomol. **132** (2008) 12–17

Pemberton 2003; Schauff 2005). But their effect on the lobate lac scale is insufficient. Pemberton (2003b) recorded only three emergence holes in 3000 scales examined.

A biological control strategy was developed involving two approaches: (1) to acquire and evaluate parasitoids of the confamilial commercially reared *Kerria lacca* (Kerr), and (2) to discover parasitoids associated with the conspecific *P. lobata* in its native India and Sri Lanka (Pemberton 2003a). Parasitoids of *K. lacca* were found in Thailand and imported to the Gainesville, Florida quarantine during 2003 and 2004, but they showed no interest in the invasive lobate lac scale and induced no parasitism (Pemberton et al. 2006). *Paratachardina lobata*, endemic to southern India/Sri Lanka below the 16th latitude (Varshney 1976a), was first described from Perideniya and Kandy in Sri Lanka, and occurs in the Indian states Tamil Nadu and Karnataka (Green 1922). During March 2004, an intensive survey for *P. lobata* was made in Sri Lanka, in Peridenyia, Kandy, and other locations, but no *Paratachardina* scales were found (R. W. Pemberton unpublished data). The research reported here began in August 2005 with a survey by Pemberton and Selvaraj in southern India. Populations of *P. lobata* were discovered at various localities in Tamil Nadu and Karnataka. Following, the emergence of parasitoids was observed from infested twigs collected and imported to the quarantine facility of the US Department of Agriculture, Agricultural Research Service (USDA, ARS), Invasive Plant Research Laboratory, Fort Lauderdale, FL. Bimonthly collections were made for 1 year at 14 sites in southern India. The present paper reports this assessment of *P. lobata* parasitoids, undertaken to discover the species attacking the scale, to define their modes of parasitism, and temporal and spatial abundance, with the goal of identifying species with potential for classical biological control of the invasive scale.

Materials and Methods

Surveys for *P. lobata* in southern India were guided by published collection localities (Varshney 1976a). *Paratachardina lobata* was located at different sites in areas around Bangalore in Karnataka and Coimbatore in Tamil Nadu. Bangalore is situated in the southeast of Karnataka, in the heart of the Mysore Plateau (a region of the larger Precambrian Deccan Plateau) at an average elevation of 920 m. Coimbatore is seated further south at the western border of the Indian state of Tamil Nadu at the foothills of Nilgiris, 330 km south of Bangalore at an altitude of 409 m above sea level. The temperature ranges in both regions from 35 to 16°C (30 years average of highs and lows). Annual rainfall reaches 800 mm in Bangalore and 600 mm in Coimbatore. Rainfall in Bangalore has two peaks (May and October), with scattered showers during the summer months. In Coimbatore the summer months have less rain and only one period of high rainfall in October to December. The temperature range is a little lower than in Bangalore (Gunnell 1997).

Paratachardina lobata collections

The scales were mainly collected from *Pongamia pinnata* (L.) Pierre (Fabaceae) except for some taken from *Magnifera indica* L. (Anacardiaceae) and *Santalum album* L. (Santalaceae) in August 2005. The collected scales were confirmed to be *P. lobata* by specialists (D.R. Miller and G.S. Hodges). From August 2005 to July 2006, six collections of *P. pinnata* twigs infested with the scale were made bimonthly. Table 1 summarizes the collection sites from north to south. The weight of one collection was about 1.5 kg, and contained samples of infested twigs from five to nine sites. Infested twigs were cut from the trees, defoliated manually, and the cut surfaces were covered with wax to prevent desiccation and to extend the longevity of the scales and the immature parasitoids within. Twigs from each collection site were double packed in bags made of jute, put into a polystyrene box with cooling packs and sent to the quarantine facility of the USDA, ARS Invasive Plant Research Laboratory in Fort Lauderdale, FL. The consignments took an average of 5 days to reach the Florida laboratory. During this time the temperature inside the package ranged from 16 to 30°C, measured by a temperature data logger (WatchDog Model 125; Spectrum Technologies Inc., Plainfields, IL, USA).

Observations on parasitoid emergence

After reception in the quarantine laboratory, the twigs from each site were stored separately in white Plexiglas cages (40 cm^3), with two transparent sides (top and front) and micromesh gauze at the back side. Water in vials sealed with saturated cotton and honey spread at the walls were provided for emerging adult parasitoids. The average *P. lobata* infestation was calculated, counting the number of scales on 3×5 cm length twig subsamples in the generated surface ($2\pi r = 5$ cm). Twigs were chosen according to scale infestation, representing the highest, medium and

Table 1 Spatial occurrence of four parasitoid species (*Coccophagus* sp., *Aprostocetus bangaloricus*, *Ooencyrtus kerriae* and *Marietta leopardina*) associated with *Paratachardina lobata* in southern India

	Collection sites					Parasitoid emergence			
	GPS coordinates								
No.	North	East	Alt. (m)	Location description (nearest city/park)	*P. lobata* 1/cm^2 (*n*)	*Cocco-phagus* sp.	*A. bangalo-ricus*	*O. kerriae*	*M. leopard-dina*
1	13°05′60′	77°53′13′	916	Bangalore, Nellali	3.2 ± 0.0 (1)	5	0	0	0
2	13°05′43′	77°51′28′	931	Bangalore, Gottipura	0.6 ± 0.0 (1)	0	0	0	0
3	13°03′34′	77°32′57′	856	Bangalore, Jarakabande	1.3 ± 0.3 (3)	16	3	4	2
4	12°58′	77°38′	1000	Malleshwaram	7.4 ± 1.1 (3)	269	24	4	0
5	12°55′68′	77°21′51′	839	Bangalore, Big Banyan Park	5.2 ± 1.1 (6)	210	73	32	2
6	12°55′63′	77°22′48′	830	Bangalore, Big Banyan Park	5.0 ± 0.0 (1)	8	18	1	1
7	12°54′68′	77°23′45′	830	Bangalore, Big Banyan Park,	1.2 ± 0.0 (1)	2	0	1	0
8	12°54′63′	77°23′45′	830	Bangalore, Big Banyan Park	1.4 ± 2.4 (4)	8	18	1	1
9	12°52′38′	77°35′69′	1111	Bannerghata National Park	6.0 ± 4.7 (2)	3	8	1	0
10	12°48′04′	77°34′61′	931	Bannerghata National Park	5.0 ± 2.8 (6)	33	131	105	20
11	12°48′00′	77°34′79′	931	Bannerghata National Park	3.9 ± 2.0 (2)	3	2	12	11
12	12°47′37′	77°37′60′	930	Bangalore, Anekal Road	0.5 ± 0.0 (1)	0	0	0	0
13	11°00′55′	76°52′00′	335	Coimbatore, Thondamuthur	5.0 ± 1.2 (6)	4	122	38	15
14	11°00′28′	76°55′22′	335	Coimbatore, Veerakeralam	2.9 ± 0.0 (1)	8	3	2	0

Scales infesting *Pongamia pinnata* twigs were collected at 14 sites bimonthly from August 2005 to June 2006, in the provinces Bangalore, Karnataka (nos 1–12) and Coimbatore, Tamil Nadu (nos 13–14). The sites are listed according to their latitude from north to south (NAD83), showing the average infestation levels of *P. lobata* and the number of times scales were collected at each site (*n*).

lowest numbers. Twig samples of sites with only a small amount of infested twigs were transferred into transparent plastic boxes (33 × 20 × 10 cm). Boxes were supplied with water and honey in the same manner. Cages and boxes were stored at 23–26°C and 65% relative humidity (RH) in a room with natural sunlight. Parasitoid emergence and the appearance of other insects were recorded at 1- to 3-day intervals for 2 months after the arrival of the shipment and then weekly for late-emerging insects during the following month. Cages /boxes were opened inside a light box to facilitate parasitoid capture using an aspirator. Emerging adult parasitoids were examined under a stereo microscope and sorted to species and gender. All specimens were preserved in 70% alcohol and sent to specialists for identification. In order to track parasitoids associated with *P. lobata*, subsamples of infested twigs were selected and cleaned of other insects and *P. lobata* with parasitoid emergence holes and placed in isolation boxes (10 × 12 × 14 cm) for observation. After parasitoids appeared in these boxes, the scales were examined to detect emergence holes and to identify the hosts. These parasitized scales were then dissected to examine remains and determine the mode of parasitism. In order to compare the size of the species, the body length of 10 females was measured using a compound microscope at 10 times magnification. Images were taken and then measured using a 14.3 3-shot colour camera (Spot advanced version 4.6; Diagnostic Instruments, Inc., Sterling Heights, MI, USA).

Data analysis

In order to compare parasitoid species associated with *P. lobata*, the size, the number of wasps emerging, as well as the time of their emergence were compared. The body length of 10 randomly chosen females of each species were measured and compared. Mean values of total number of emerged specimens per collection period were analysed for each species. The mean day of emergence for each species was calculated, by the number of days from the date of collection to the date of emergence of each individual. The six collection periods were counted as replicates. Data were analysed, using the Bonferroni test of ANOVA (SPSS 10.0; SPSS Inc., Chicago, IL, USA). The significant levels were at 5%.

Results and Discussion

The microcosm of pongam twigs collected in India is very rich in arthropod diversity. In addition to heavy infestations by *P. lobata* (up to 20 scales/cm), various other scales such as coccids, pseudococcids, diaspidids and margarodids were found. Four mite species, bark beetles, ants of five genera, thrips, bark lice, lacewings and a pseudoscorpion were associated

J. Appl. Entomol. **132** (2008) 12–17

with the twigs. Some of these arthropods were parasitized, resulting in a great number of various chalcidoid wasp species. From the twig collections, 26 parasitoid species emerged but only four proved to be associated with *P. lobata*: a eulophid *Aprostocetus bangaloricus* Narendran, an encyrtid *Ooencyrtus kerriae* Hayat and two aphelinids *Marietta leopardina* Motschulsky and an unnamed *Coccophagus* sp. of the ochraceus group, very close to *longiclavatus* Shafee (Hayat) (M. Hayat personal communication). *Aprostocetus bangaloricus*, *O. kerriae* and *M. leopardina* are already specified by Hayat (1997) and Hayat et al. (2003).

Marietta leopardina (Aphelinidae)

Marietta leopardina is known to attack diverse scales including *P. lobata* (Noyes 2003). This minute parasitoid has a body length of 0.6 (±0.08) mm, significantly smaller than *O. kerriae* and *A. bangaloricus* ($F_{3,36} = 13.048$, $P < 0.001$). This species occurred in low numbers in our study. Subsample dissections of attacked host scales indicated that this parasitoid emerged from immature scales of both gender ($n = 5$) and mature females ($n = 2$). No parasitoid remains were found inside these scales, indicating that this species is a primary parasitoid of *P. lobata*. However, *M. leopardina* is known to be a secondary parasitoid of a great number of chalcidoid wasps (Hayat et al. 2003; Noyes 2003). Pemberton (2003b) listed the species as a potential hyperparasitoid of chalcid wasps attacking *K. lacca*. Hence it has no potential as a biological control against the lobate lac scale in Florida or the Bahamas.

Coccophagus sp. (Aphelinidae)

Aphelinids can have interesting and various parasitic habits. For example, autoparasitism occurs in which males develop as adelphoparasitoids, or females and males can develop in different hosts (Viggiani 1984; Hayat 1997). Subsample dissections indicated that the *Coccophagus* sp. associated with *P. lobata* emerged from mature ($n = 15$) as well as from immature scales ($n = 5$). Body length was 0.7 (±0.09) mm, significantly smaller than *O. kerriae* ($F_{3,36} = 13.048$, $P < 0.001$), but similar to the other parasitoids associated with *P. lobata*. The occurrence of males was very rare (≥16 ♀ : 1 ♂), which is quite common for aphelinids (Viggiani 1984). No evidence for autoparasitism or sexual difference in host preferences was observed. Another member of the same genus *Coccophagus*, *C. tschirchii*, was found to be a primary parasitoid of *K. lacca* (Varshney 1976b; Subbarayudu and Maheswahar 1998; Pemberton et al. 2006). However, the *Coccophagus* species we obtained was found to be a secondary parasitoid of *P. lobata*. Dissected scales contained the last tergites of other parasitic wasp species. These parasitoid remains were fully developed exoskeletons of dark colour, indicating koinobiontic development. The average emergence time after the collection date was with 41 days significantly greater than for the other parasitoids ($F_{3,19} = 10.1$, $P \leq 0.037$), presumably due to hyperparasitism, because the primary host needed to develop first. The abundance of *Coccophagus* sp. was, with a mean of 90 wasps, relatively high (table 2). This species was just described as *C. parlabatae* Hayat (Hayat 2007)

Ooencyrtus kerriae (Encyrtidae)

This species is placed in the genus *Ooencyrtus* because it has many morphological characteristics of this genus, but some characteristics of this species such as antennal scropes are unique (Hayat et al. 2003). In this study it was found to emerge from mature female *P. lobata* scales ($n = 18$). Evidence for superparasitism was found in subsample dissections with up to four remains of fully developed *O. kerriae* adults within single scales ($n = 12$). But single emergence holes and no remains ($n = 6$) or multiple holes in the same empty scale remains were also found ($n = 4$), indicating gregarious as well as solitary development in this species. The mean body length of *O. kerriae* was 1 (±0.2) mm and some individuals were only half as big as others. The great size variation is probably due to the variation between number of wasps developing inside one scale. The

Table 2 Chalcidoidae associated with *Paratachardina lobata* from six collection periods of infested *Pongamia pinnata* (Fabaceae) twigs from collections at 14 sites in southern India, August 2005 to June 2006 (Table 1)

Species	Average number of emerged wasps per collection period (±SD)	Average days to emergence after collection (±SD)	Sex ratio (*n*) ♀ : ♂
Coccophagus sp.	90.5 (87.3) a	41 (4.3) b	≥16 : 1 (402)
Aprostocetus bangaloricus	62.8 (37.1) a	20.8 (3.4) a	1 : 1 (372)
Ooencyrtus kerriae	31.7 (36.0) a	28 (11.3) a	2 : 1 (49)
Marietta leopardina	11.9 (9.1) a	20.3 (3.6) a	3 : 1 (190)

Mean values ($n = 6$) within a column followed by the same letter do not differ significantly according to Bonferroni test at $P < 0.05$.

mean number of emergence per collection (approximately 1.5 kg of twigs) was 32 wasps, which was a relatively low number (table 2).

Aprostocetus bangaloricus (Eulophidae)

This is one of 43 species in the genus *Aprostocetus* Westwood recorded from India (Hayat et al. 2003). The congeneric species *A. purpureus* is another parasitoid recorded from lac insects (Varshney 1976b). This species is the most abundant parasitoid recorded from *K. lacca*, but it is a facultative hyperparasitoid (Subbarayudu and Maheswahar 1998; Pemberton et al. 2006). In our study *A. bangaloricus* emerged from immature *P. lobata* of both genders ($n = 6$) as well as from mature female scales ($n = 13$), determined by subsample dissections. The absence of parasitoid remains inside dissected parasitized scales ($n = 18$) indicated primary, solitary development. The wasps measured 0.9 ($\pm$1) mm in body length, and size of individual wasps varied (as in *O. kerriae*), but this variation is probably due to the different developmental host stages. Mean emergence per collection period was 63 wasps, which was relatively high (table 2). *Aprostocetus bangaloricus* occurred in lower numbers than the *Coccophagus* sp., but its incidence was more constant (fig. 1). The number of emerging wasps per collection period was synchronous with the number of emerging *O. kerriae*, but consistently higher. The impact of *A. bangaloricus* on lobate lac scales might be greater than that of *O. kerriae*, because of its higher abundance, exclusively solitary development, and tendency to attack earlier stages of the pest.

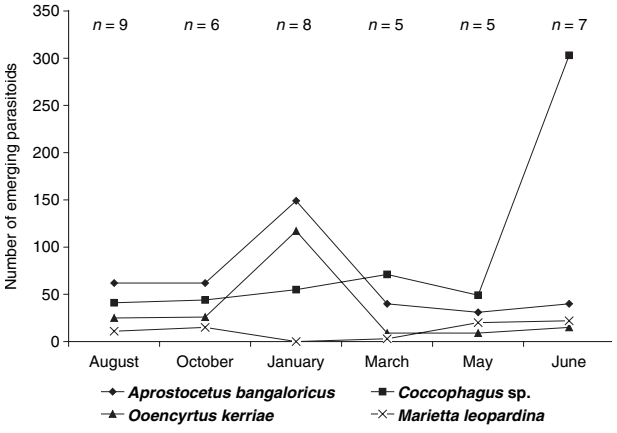

Fig. 1 Chalcidoid species associated with *Paratachardina lobata* from six collection periods. Collections of *Pongamia pinnata* (Fabaceae) infested with the scale were made bimonthly at five to nine sites from 14 sites in southern India, August 2005 to June 2006 (table 1).

Spatial occurrence

The parasitoids associated with *P. lobata* were observed from almost every collection site (table 1). They were not obtained from collections in Bangalore, province of Karnataka at Gottipura (no. 2) and Anekal Road (no. 12), which might be due to the low numbers of *P. lobata* at these sites. From Nellali, Karnataka (site no. 1) only five specimens of *Coccophagus* sp. emerged, and *A. bangaloricus* was absent from one collection site at Big Banyan Tree Park, Karnataka (no. 7). The detection of the four parasitoids at all other sites indicates that these antagonists occur widely in southern India. *Paratachardina lobata* was most abundant in Karnataka at the Malleshwaram Circle (site no. 4), on the road to Big Banyan Tree Park (site no. 5), at the Bannerghata National Park Road near Bangalore (site no. 10) and at Thondamuthur Road in Coimbatore in Tamil Nadu (site no. 13) (table 1). More parasitoids were recovered where the scale was highly abundant. The *Coccophagus* sp. was most abundant in Karnataka at Malleshwaram Circle (no. 4) and on the road to Big Banyan Tree Park (no. 5). At these sites, 299 *Coccophagus* sp. emerged from a single collection period. *Aprostocetus bangaloricus* and *O. kerriae* were also abundant at these sites. However, the greatest numbers for *A. bangaloricus*, *O. kerriae* and *M. leopardina* were recorded from the Bannerghata National Park Road near Bangalore, Karnataka (site no. 10) and Thondamuthur Road in Coimbatore, Tamil Nadu (site no. 13).

Temporal occurrence

More than 300 *Coccophagus* wasps emerged from the June collection, compared with 41–71 emerging wasps in other collection periods (fig. 1). *Aprostocetus bangaloricus* was abundant at almost every site and at each collection period, but it was most abundant in January, when *O. kerriae* was also the most numerous. Variation in parasitoid abundance may have been due to climatic conditions. Heavy rains have an adverse effect on adult mortality and oviposition activity (Fink and Voelkl 1995). The number of parasitoids might increase during the drier seasons after the rain peaks in May and October to December. This may have promoted the abundance of primary parasitoids during the drier season in January and subsequently the development of the secondary parasitoid later, which emerged during the 'little dry season' in June.

J. Appl. Entomol. **132** (2008) 12–17

Future research

Compared with the great number and abundance of parasitoids of lac insects recorded in the literature (Varshney 1976b; Pemberton 2003b; Pemberton et al. 2006), the number of species in this study is relatively low. Some of the most important parasitoids associated with *K. lacca* reach parasitism levels of up to 50%. Further collections including ones of different host plants and collection areas, e.g. in the neighbouring Indian states Kerala, Andhra Pradesh, as well as in Sri Lanka, might increase the number of parasitoid species associated with the lobate lac scale. *Aprostocetus bangaloricus* and *O. kerriae* were primary parasitoids and the *Coccophagus* sp. a facultative primary parasitoid of *P. lobata* found in the present investigation. Determining their ability to parasitize the invasive pest lobate lac scale and defining their host ranges will be the focus of quarantine evaluations, which will help judge their suitability for use in Florida and the Bahamas.

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